EFFECT OF PH AND TEMPERATURE ON FRUCTOSYLTRANSFERASE (FTase) PRODUCTION FROM *CANDIDA KRUSEI*

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Thesis submitted to the Faculty of Chemical and Natural Resource Engineering in fulfillment of the requirements for the award of the Degree of Bachelor of Chemical Engineering in Biotechnology

Faculty of Chemical and Natural Resources Engineering
UNIVERSITI MALAYSIA PAHANG

FEBRUARY 2013

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ABSTRACT

The relationship between consumer, food and health become one of important issues because of increasingly demand on healthier food. Based on functional properties of fructosyltransferases (FOS), the consumer demands have been increasing in order to response on that. In Malaysia, it is reported that the number of diabetic patients is increasing daily and it is one of the fatal diseases. A significant driving force in the 'functional food' market place is the quest by consumers to optimize their health through food. The enzyme source synthesis production can be divided into two classes which are from microorganism and plant. Where by plant, the production is restricted from any sources .In view of that, production of crude enzyme from micro fungi is studied in order to obtain high yield of FOS. This study is carried out using sucrose as a substrate in the fermentation process to produce crude enzyme from micro fungi, namely *Candida krusei*. In this study, an investigation on pH and temperature of enzyme was analysing ranging from 3.0-8.0 and 25-55^{o}C, respectively. The crude enzyme was analysed using UV-Vis U-1800 Spectrophotometer at wavelength of 540 nm. The protein concentration was determined using UV-Vis U-1800 Spectrophotometer at wavelength of 750 nm. By using an extracellular enzyme which is fructosyltansferase (FTase) from selected micro fungi, the food-grade FOS can be produced commercially from sucrose. As result indicates that the highest pH and temperature for activity of FTase from *Candida krusei* is pH5.5 and temperature 55^{o}C. The highest enzyme activity (148.501 IU/mL) was figured out at pH 5.5, 55^{o}C and the specific activity was observes as 10.638 IU/mg. As a conclusion, this research was done to produce other alternative way of producing crude FTase using selected microfungi that are less harmfull to use in a production of FOS in food health industries.

KESAN DARI PENGGUNAAN PH DAN SUHU TERHADAP PENGHASILAN FRUKTOSILTRANSFERASE (FTase) DARI *CANDIDA KRUSEI*

ABSTRAK

Hubungan yang terjalin antara pengguna, makanan dan juga kesihatan menjadi salah satu isu yang penting kerana meningkatnya permintaan terhadap makanan yang sihat. Berdasarkan kepada ciri-ciri dan fungsi yang ada pada fruktosiltransferase (FOS), permintaan pengguna terhadapnya telah meningkat. Di Malaysia, telah dilaporkan bahawa bilangan pesakit kencing manis semakin meningkat setiap hari dan ia merupakan salah satu penyakit yang membawa maut. Satu daya penggerak penting dalam pasaran "makanan berfungsi" adalah usaha yang dilakukan oleh pengguna untuk mengoptimumkan kesihatan mereka melalui pemilihan makanan yang betul. Sumber penghasilan sintesis enzim boleh dibahagikan kepada dua kelas iaitu mikroorganisma dan juga tumbuhan. Jika dari tumbuhan, pengeluaran enzim adalah terhad dari mana-mana sumber. Sehubungan itu, pengeluaran enzim mentah dari kulat mikro telah dikaji dalam usaha mendapatkan penghasilan yang tggi dalam FOS. Kajian ini telah dijalankan menggunakan sukrosa sebagai substrat dalam proses penapaian untuk menghasilkan enzim mentah dari kulat mikro, iaitu *Candida krusei*. Dalam kajian ini, siasatan telah dijalankan keatas kesan pH dan suhu enzim yang telah dianalisis masing-masing antara 3.0-8.0 dan 25-55ºC. Enzim mentah dan kepekatan protein telah dianalisa dengan menggunakan UV-Vis U-1800 Spektrofotometer pada panjang gelombang 540 nm dan 750 nm. Dengan menggunakan enzim extracellular fruktosiltransferase (FTase) daripada kulat mikro yang dipilih, gred makanan berasaskan FOS boleh dihasilkan secara komersial daripada sukrosa. Hasil keputusan menunjukkan bahawa pH dan suhu paling tinggi untuk aktiviti FTase enzim dari *Candida krusei* adalah pada pH 5.5 dan suhu 55ºC. Aktiviti enzim yang tertinggi (148.501 IU/mL) telah dicatatkan pada pH 5.5, 55ºC dan aktiviti tertentu telah diperhatikan pada nilai 10.638 IU/mg. Sebagai kesimpulannya, kajian ini dilakukan untuk menghasilkan cara alternative lain dalam menghasilakn FTase mentah menggunakan mikrofungi terpilih yang kurang berbahaya untuk digunakan dalam penghasilan FOS dalam industri makanan kesihatan.

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LIST OF SYMBOL

GF_2	1-kestose
GF_3	nystose
GF_4	1-β-fructofuranosylnystose
g/L	gram over liter
w/w	weight over weight
w/v	weight over volume
IU	1 unit of enzyme

LIST OF ABBREVIATION

US	United Stated
BSA	Bovine serum albumin
DNS	Dinitrosalicyclic acid
FFase	Fructofuranosidase
FFT	Fructan-1-fructosyltransferase
FOS	Fructooligosaccharides
FOSHU	Food of Specified Health Used
FTase	Fructosyltransferase
OD	Optical density
YEA	Yeast extract agar
SST	Sucrose 1-fructosyltransferase

CHAPTER 1

INTRODUCTION

1.1 Background of Study

The global market of functional foods is estimated up to 33 billion U.S dollar. It was generated from large country such as United Stated, Japan and among European countries like Germany, France, the United Kingdom and Netherlands. Specific health related food category called FOSHU (Foods of Specified Health Use) in Japan is one another important market of functional foods in industries. According to Sangeetha *et al.*,(2005) said that an estimated market value of FOSHU labelled products was 2 billion U.S dollar in year 2000 with an estimated turnover of 14 billion US dollar. An estimated market value of FOSHU labelled products was 2 billion US dollar in year 2000 with an estimated turnover of 14 billion US dollar (Sangeetha *et al*., 2005) .

Yet, various forms of functional foods have already been introduced into the market in a large quantity which contains a number of characteristic functional ingredients such as include dietary fiber, oligosaccharides, sugar alcohols, peptides and proteins, prebiotics and probiotics, phytochemicals and antioxidants and polyunsaturated fatty acids (Sangeetha *et al.*, 2005).

Dietary carbohydrates have been caught a lot of attention especially carbohydrates, in particular, fructooligosaccharides (FOS). Their popularity has been increased as food ingredients because of the possible health beneficial associated with the consumption of these compounds and also being promoted as alternative sweeteners for diabetic formulations. According to Flamm *et al.*, (2001) average daily consumption of FOS has been estimated to be 1–4 g in US and 3–11 g in Europe. The most common sources of FOS are wheat, honey, onion, garlic and banana (Flamm *et al.*, 2001).

For the past two decades, research work has been carried out all over the world on the production, properties, analytical aspects and nutritional benefits of FOS. Many review articles describing the occurrence, preparation, properties and applications of FOS (Crittenden & Playne, 1996; Yun, 1996; Slavin, 1999) have been published. Flamm *et al.* (2001) have critically reviewed the composition and source of FOS, its physiological effects upon consumption, and its relation to the dietary fibre concept.

The production of fructooligosaccharides (FOS) has received particular attention in recent years because of their excellent biological and functional properties and necessities on the development of efficient enzymatic systems. FOS posses extraordinary importance as functional food ingredients owing to their prebiotic properties (Sangeetha *et al.*, 2005).

FOS is composed of 1-Ketose (GF2), nytose (GF3) and F-fructofuranosyl (GF4) in which fructosyl units are linked at β-2,1 positions of sucrose. Neo-frustooligosaccharides (neo-FOS) with β-2,6 linkage between two fructose units (6F-FOS; 6-Ketose) or between fructose and a glucosyl group (6G-FOS; neo Kestose, neo nystose and neo-fructofuranosylnystose) have a structure different from that of FOS and might have better prebiotic properties and chemical stability compared to FOS (Chen *et al.*, 2010).

FOS production at industrial scale is commercially produced using two classes of enzymes either microbial fructosyltransferases or β-fructofuranosidases also called invertases. The enzyme source synthesis (FTase) can be divided into two classes which are from plants and microorganisms. FTase is produced intra- and extracellular by several fungi.

The enzymes source of FOS synthesis or known as fructosyl-transferring enzymes have been purified and characterized into two classes; one is higher plants such as asparagus roots, onion bulbs, Jerusalem artichoke; and the other one consists of different organisms which are bacteria and fungi such as *Aspergillus niger, Aspergillus japonicus, Bacillus macerans, Schwanniomyces occidentalis*, *Candida utilis* (Ghazi *et al.*, 2007) *Aureobasidium* sp., *Aspergillus* sp., *Arthrobacter* sp. and *Fusarium* sp. (Park *et al.*, 2001).

1.2 Problem Statement

Many bioproducts are enzymes derived from plant, animal or microorganisms and there is a great demand for their production. Fructosyltransferase is one of the enzymes that are being derived from microorganisms such as *Aspergillus niger*, *Aspergillus japonicus*, *Bacillus macerans*, *Schwanniomyces occidentalis*, *Candida utilis* and *Candida krusei* (Ghazi *et al.*, 2007).

Purification and preliminary characterization of this enzyme from various sources have been reported. However, until now the accumulated information on fructosyltransferase is rather confusing differing from one source to another, from one microorganism to another, even from one strain to another. The source of enzyme and the difficulty of purification no doubt influenced the results obtained and thus the conclusion drawn. The purification and the characterization of this enzyme are necessary steps to improve our understanding of its mode of action, the nature of the hydrolytic activity and to decide the type of enzyme in which it should be classified (L'Hocine *et al.*, 2000).

Most of enzyme produced from microbial such as bacteria and fungi are harmful to human health. Thus, this study was proposed to solve that problem by using less harmful microbial which is *Candida krusei* by doing study on the enzyme production. By using this microbial behaviour, it can improve the fructosyltransferase production by using nature materials which is less harmful, reduce the production cost and make it more environmental friendly.

This research was designed to determine the optimum condition of temperature and pH in fermentation broth to acquire the highest amount of fructosyltranferase produce by chosen microbe. Thus, the target of this research to study the production of fructosyltransferase enzyme by using *Candida krusei*.

1.3 Research Objectives

The main objective of this research is to study the effect of pH on enzyme production from *Candida krusei* using sucrose as a substrate

The measurable objectives of this research are:

a) To determine the effect of incubation time of fructosyltransferase production using *Candida krusei*.
b) To study the effect of temperature on crude enzyme produced from *Candida krusei*
c) To study the effect of pH on crude enzyme produced from *Candida krusei*
d) To determine the activity and specific activity of enzyme produced from *Candida krusei*

1.4 Scope of Study

FTase produced from microfungi derived from sucrose is more preferable. In view of that, production of crude enzyme from microfungi is studied in order to obtain high yield of FTase. This study is carried out using sucrose as a substrate in the fermentation process to produce crude enzyme from microorganism. This study is beginning with the cultivation of the enzyme. The microorganism is maintained on agar petri plate at 4^oC. The inoculums is developed by transferring the mycelia from a 3 days old agar petri plate into the inoculum medium consist of (g/l) 10 sucrose, 3 yeast extract at pH 5.5. The flask was incubated for 24 h at 30^oC on a rotary shaker.

After 24 h, the inoculum was transferred into 250 ml of fermentation medium consisting of (g/l) 30 sucrose, 5 yeast extract, 3 peptone, 0.5 magnesium sulphate heptahydrate and 0.5 kalium phosphate. The inoculum will undergo fermentation and transform into biomass cell. Then it will be separated to obtain the supernatant which is source of enzyme. The enzyme is assayed with different temperature, pH and incubation time range in order to determine the effect of it towards enzyme activity produced *by Candida krusei*. Then, the activities of crude enzymes and protein concentration is analysed by using correlation of glucose standard curve from UV-Vis Spectrophotometer for the enzymatic activities.

1.5 Rationale and Significance

Fructosyltransferase (FTase) enzymes were widely used as a biocatalyst in the production of fructooligosaccharides (FOS) in many food and beverage industries. There are a lot of sources with fructosyltransferase enzyme production in industries nowadays. These researches were conducted in order to improve the amount of fructosyltransferase which produce by chosen bacteria such as *Candida krusei*. Moreover, the rationale of this paper to determine the optimum conditions of fructosytransferase production by monitoring parameters such as time and pH used. As known, fructosyltransferase can be produced from chemical and microbial production. This microbial techniques were resulted with reduces the cost production of fructooligosaccharides and increase the demand on manufacturing using this technique. It also was more feasible, economical, environmental friendly and convenient suit to enzyme and biotechnology field.

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

The production of fructooligosaccharide (FOS) has been an interest at the industrial level for the world market. Mainly, the microbial production of FOSs is enzymatic reaction has been selected rather than chemical synthesis due to acknowledge as being more feasible, economical, environmentally friendly and more convenient. According to research done by sangeetha et al. (2005) said that basically this method requires the two stage experimental work. The first stage refers to the production of enzyme which is fructosyltransferases (FTase) from microbes and finally FTase will be used for the second stage that is reaction with sucrose to produce FOS.

In this study, the enzymatic reaction will be remaining but the process of microbial production of FTase will be focused more as an objective. Besides, the fungal *Aspergillus niger* and the yeast of *Candida krusei* species were also has been introduces as the new sources of FTase production. These approaches will be studied and discussed in this research for the new technique beyond industrial application for FTase production that know is really important in human's health and life.

2.2 Sucrose

Sucrose is one of the organic compounds that are widely known as table sugar and sometimes called saccharide. It has physical properties such as white, odourless, crystalline powder with a sweet taste. The best features of sucrose commonly known as best in nutritional role. It is produced naturally by all plants as food by combining glucose and fructose molecules during the process of photosynthesis with the molecular formula $C_{12}H_{22}o_{11}$ (Klaus *et al.*, 2002).

The sucrose molecule falls into multiple biochemical classes. It is a carbohydrate, meaning that it's composed of carbon, hydrogen and oxygen. More specifically, it is a small, sweet-tasting carbohydrate. Sucrose is composed of two smaller sugar units, specifically glucose and fructose. Sucrose is quite common in nature, occurring in many fruits and other plant parts (Klaus *et al.*, 2002).

2.3 Oligosaccharides

Oligosaccharides are carbohydrates which have 3-10 simple sugars linked together. They are found naturally, at least in small amounts, in many plants. Plants with large amounts of oligosaccharides include chicory root, from which most commercial inulin is extracted, and so-called Jerusalem artichokes (the root of a member of the sunflower family). They are also found in onions (leeks and garlic), legumes, wheat, asparagus, jicama, and other plant foods (Maiorano *et al.*, 2008). Most of oligosaccharides have mildly sweet taste, and have certain other characteristics, such as the mouth feel they lend to food, that has drawn the interest of the food industry as a partial substitute for fat and sugars in some foods as well as improved texture. Because of this, more and more of the oligosaccharides in food synthetically produced. Recent interest has also been drawn to oligosaccharides from the nutritional community because of an important characteristic: the human digestive system has a hard time breaking down many of these carbohydrates (Laura Dolson, 2009).

2.4 Fructooligosaccharides (FOS)

Fructooligosaccharides (FOS) also known as oligofructose or oligofructan used as alternative sweetener. Fructo-oligosaccharides are made up of plant sugars linked in chains. FOS exhibits sweetness levels between 30 and 50 % of sugar in commercially-prepared syrups. Its molecular structure of GF-Fn, (n = 1-9). They are most taken from asparagus, Jerusalem artichokes, and soybeans. It occurs naturally, and its commercial use emerged in the 1980s in response to consumer demand for healthier and calorie-reduced foods (Noraziah *et al.*, 2011). People use this sugar to make medicine and known as nutrition, health care, the health effects trinity of the twenty-first century, a new sugar source (Noraziah *et al.*, 2011).

FOS is an important composition of human diet. It has significant effect to improve bowel function, prevent constipation and diarrhea, lower blood lipids and the human immune system. It is because fructooligosaccharides having a wide variety of physiological functions and superior physical and chemical properties.

2.4.1 Physical and Chemical Properties

FOS are about half as sweet as sugar but with a fraction of the calorific value and as they were first developed as a low calorie, low cariogenic, sugar substitute. However, as awareness has grown of the differential effects that FOS has on the human microflora, it has become the standard bearer of ‘prebiotics’ as a new type of nutritional supplement. According to the Zhengzhou Friend Biological Engineering Co., Ltd stated that FOS was having a variety of physical and chemical properties and also the health indicator as shown in Table 2.1.

Table 2.1 Physical and chemical properties of FOS

Item	Indicators
Physical	
Solid (20 refractive count method)%≥	75
Total content%≥	50,70,90
Chemical	
PH Value	4.5-7.0
Arsenic (As)mg/kg≤	1.0
Lead(Pb)mg/kg≤	0.5

(Source: Zhengzhou Friend Biological Engineering Co., Ltd)

2.4.2 Functional Properties of FOS

Regular use of fructooligosaccharides can cause health effect to users. This has been proven through research study by Sangeetha *et al.*, (2005). Studies with inulin and FOS have shown reduction of chemically induced aberrant crypts and prevention of colon cancer. According to Pool-Zobel *et al.*,(2002) in rats, a prebiotics effect resulting in the proliferation of bifidobacteria (with major metabolites lactate or acetate) as well as of other bacteria could be responsible for the observed anticancer effects. According to Henryk & Marcel (2005), dietary treatment with inulin/oligofructose (15%) incorporated in the basal diets for experimental animals resulted in (a) reduction of the incidence of mammary tumors induced in Sprague Dawley rats by methylnitrosourea (b) inhibited the growth of transplantable malignant tumors in mice and (c) decrease the incidence of lung metastases of a malignant tumor implanted intramuscularly in mice. It is reported that the dietary treatment with FOS/inulin significantly potentiated the effects of subtherapeutic doses of six different cytotoxic drugs commonly utilized in human cancer treatment (Sangeeta *et al.*, 2005).

2.4.3 Application of Fructooligosaccharides

There have been reported that FOS was used in many application such as medical, human defences system, and metabolism and also food formulations were using FOS as one of ingredient in the products. It makes fructooligosaccharides become as a target point in the many research nowadays even a long time ago because of their potential to become dietary carbohydrate that are also being promoted as alternatives sweetener for diabetic patience. Table 2.2 indicates some of the application involve with FOS.

Table 2.2 Application of FOS

Application	Description	References
Prebiotic	-Investigation have been performed that the prebiotic of FOS by studying the metabolism of two types of chicory fructooligosaccharides by *Bifidobacterium longum, B. infatis and B. angulatum.* Biomass production was highest with *B. infatis* (1.4 and 1.7 g dry wt/l) for it cultivation in a medium supplemented with fibruline instant as substrate	Durieux *et al.*, (2002)